Conifer root nodules colonized by arbuscular mycorrhizal fungi in Jurassic geothermal settings from Patagonia, Argentina

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ABSTRACT

Premise of the Research: Despite their ecological significance in modern terrestrial ecosystems, knowledge about the evolution of arbuscular mycorrhizae based on the fossil record is still scarce, especially concerning the case of root nodules harboring arbuscular mycorrhizal fungi as in some extant gymnosperms and angiosperms. Exceptionally preserved conifer nodular roots were found in the Jurassic fossil-bearing chert deposits of the Deseado Massif (Santa Cruz, Argentina), raising the possibility to study them in association with arbuscular

mycorrhizal fungi. The aim of this study is to describe the plant organs and their fungal partners, and to discuss the ecological significance of the interactions observed, particularly with respect to their occurrence in the hot-spring settings.

Methodology: Thin sections of chert samples from the 'Cañadón Nahuel' locality of the La Matilde Formation, Deseado Massif (Santa Cruz, Argentina) were observed using light microscopy.

Pivotal Results: The cortex of the nodules are occupied by several glomeromycotan fungal structures. The structures occur in a specific zone of the cortex - towards its center -, and includes intracellular hyphal coils and arbuscules. Glomoid spores, and coenocytic hyphae possibly penetrating the epidermal cells are also described and analyzed.

Conclusions: The root nodules have affinities with the Araucariales, representing the oldest record of such structures for this conifer clade. This is also the first record nodules harboring arbuscular mycorrhizal fungi for the Jurassic; it extends our knowledge of the fossil record of this particular type of fungal association.

Keywords

Root nodules; arbuscular mycorrhizal fungi; hot-spring settings; Araucariales; Jurassic

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) have a deep fossil record (Pirozynski and Dalpé 1989; Remy et al. 1994; Taylor et al. 2015; Walker et al. 2018 and citations therein) and today develop symbiosis with approximately 80% of vascular plants worldwide, including five major groups of living conifers (Brundrett 2004; Smith and Read 2008; Spatafora et al. 2016; Wang and Qiu 2016; Brundrett and Tedersoo 2018). In modern conifers, AMF colonize roots of the Taxaceae, Cupressaceae, Sciadopityaceae, Araucariaceae and Podocarpaceae (Brundrett 2009; Wang and Qiu 2006; Dickie and Holdaway 2000). The members of the Araucariales clade (*i.e.* members of the Podocarpaceae and Araucariaceae) and *Sciadopytis* harbor the AMF in young fine roots and small spherical differentiated structures called nodules (Spratt 1912; Godoy and Mayr 1989; Breuninger et al. 1999; Mcgee et al. 1999; Russell et al. 2002; Leone et al. 2008; Dickie and Holdaway 2011; Padamsee et al. 2016). Although AMF are relatively well-known from the fossil record (Taylor et al. 2015; Strullu-Derrien et al. 2018; Walker et al. 2018), the fossil record of arbuscular mycorrhizal associations with conifers and related groups is sparse.

Structurally preserved AMF have been described in Carboniferous Cordaites from the Massif Central, France (Strullu Derrien et al. 2009), in voltzialean conifers from the Triassic of Antarctica (Schwendemann et al. 2011; Harper et al. 2015), and in a taxodiaceous Cupressaceae from the Middle Eocene of Canada (Stockey

et al. 2001). Nodular roots related to the Araucariales are known from the Cretaceous of Antarctica, Australia and India (Cantrill and Douglas 1988; Cantrill and Falcon-Lang 2001; Banerji and Ghosh 2002), and in the Triassic voltzialean *Notophytum krauselli* (Schwendemann et al. 2011, Harper et al. 2015). This last record is the only one showing anatomical preservation of the rootlets in which AM fungal colonization has been described.

The Middle-Late Jurassic chert deposits of the Bahía Laura Group in the Deseado Massif of Santa Cruz Province, Southern Patagonia, Argentina bear exceptionally preserved organisms in hot-spring settings (Channing et al. 2007; Guido et al. 2010; Channing et al. 2011; García Massini et al. 2012, 2016). The best preserved Jurassic geothermal paleosurfaces can be positively compared based on their structure to hot springs at Yellowstone National Park in the United States (Channing et al. 2009a,b). These are the only geothermal fossiliferous deposits with an ecosystem-level preservation known for the Mesozoic. Preliminary reports on the fossiliferous content and paleoenvironment reconstruction have been carried out on a few localities (Channing et al. 2007; Guido et al. 2010; García Massini et al. 2012; García Massini et al. 2016; Sagasti et al. 2016). Plant remains and fungal and fungus-like vegetative and reproductive structures are abundant (Guido et al. 2010, García Massini et al. 2012, García Massini et al. 2013; García Massini et al. 2016, Sagasti et al. 2016, Sagasti et al. 2019), and frequently in association with each other (*e.g.* García Massini et al. 2016). This provides the opportunity to describe a variety of plant-fungal interactions to characterize the dynamics of the hot-springs associated ecosystems and to

broaden our knowledge of fungal evolution and diversity during the Jurassic, and in a broader sense during the Mesozoic.

In this contribution we describe particular conifer nodular roots colonized by AM fungi, preserved as inclusions in the matrix of chert blocks collected from the recently discovered 'Cañadón Nahuel' locality of the La Matilde Formation, Bahía Laura Group, at the Deseado Massif hot-spring deposits. We discuss possible systematic affinities for these roots based on comparisons with extinct and extant examples of conifer nodular roots. We also address the ecological significance of the arbuscular mycorrizhal interaction between the studied conifers and glomeromycotan fungi in the context of the hot-springs settings. This finding expands our knowledge on fungal diversity and ecology in the Jurassic and fills a gap in the fossil record of arbuscular mycorrhizal symbiosis in conifers.

MATERIALS AND METHODS

Geological setting

The studied samples were collected at the 'Cañadón Nahuel' locality of the La Matilde Formation, in the Bahía Laura Complex of the Deseado Massif, Santa Cruz Province, Argentina (Fig. 1). The Deseado Massif is an extensive (60,000 km^2) region in southern Patagonia, in Santa Cruz Province, Argentina. The geologic history of this region is marked by volcanic activity during the Jurassic. Approximately 178-151 million years ago (Pankhurst et al. 2000), this area was subject to bimodal rhyolitic and andesitic volcanism, which lead to the formation of the Bahía Laura Complex (Guido 2004). Rocks of the Bahía Laura Complex are part of the Chon Aike Silicic Large Igneous Province (which ranges from Patagonia

115 to Antarctica; Pankhurst et al. 1998). This province's genesis is related to the
116 break-up of Gondwana and birth of the South Atlantic Ocean (Riley et al. 2001;
117 Richardson and Underhill 2002).

118 Widespread hydrothermal activity in the Deseado Massif resulted from the
119 mature (quiescent) volcanic phase in this region during the Late Jurassic, which
120 produced metalliferous epithermal deposits (i.e. formed at shallow depths related
121 to the terrestrial surface, in the upper portion of a hydrothermal system under
122 temperatures between 150 and 300 ^{o}C), extensive silicification, and formation of
123 geothermal fields at the surface (Schalamuk et al. 1997; Guido and Campbell
124 2011). Well-preserved fossilized geothermal fields have been found within
125 volcaniclastic fluviolacustrine strata, distributed in a 400 x 250 km area of NNW-
126 SSE and WNW-ESE oriented belts aligned with major regional structures (Guido
127 and Campbell 2011). Twelve of these geothermal fields preserve fossil
128 assemblages, which represent the only known fossiliferous Mesozoic hot-springs.
129 These deposits fill a significant stratigraphic gap between similar Paleozoic and
130 Cenozoic deposits (Channing and Edwards 2013).

131 'Cañadón Nahuel' (GPS coordinates available upon request) is the farthest
132 east of these chert deposits in the Deseado Massif (Fig. 1). The locality is located
133 in the Northern Belt defined by Guido and Campbell (2011). Cherts from 'Cañadón
134 Nahuel' are rather randomly distributed over a large area and show textural and
135 fossiliferous characteristics that relate them to formation in the distal parts or
136 fringes of a siliceous sinter geothermal system. The observed distribution of the
137 cherts suggests they have been successively removed by recurrent volcanic and
138 phreatic local eruptions or breccias (i.e. by fragmentation) related to the

geothermal activity. In some instances, horizontal bed cherts of variable extension are found and these too are interpreted as having formed in wetlands and shallow saline ponds, in the distal parts of geothermal systems.

Preparation and imaging of studied materials

Thin sections of chert blocks were prepared following standard methodology (Jones and Rowe 1999). Slides were observed using transmitted light microscopy. All images were taken with a Nikon DS-Fi1 camera connected to a Nikon Eclipse 80i microscope. General views of the sections were taken using a Canon 7D camera with a Canon macro lens of 60 mm, in a light box with fluorescent tubes. At higher magnifications, multiple images of the same structure of interest were taken at different focal planes to compose an image with greater depth of focus, following an image-stacking technique (Bercovici et al. 2009). We used Helicon Focus software (http://www.heliconsoft.com/heliconfocus.html), with the ‘‘Method B’’ (Depth Map) parameter. Several partially overlapping images were merged to create high-quality images of critical features. This technique was applied using Adobe Photoshop CS6, and images were processed after to reveal critical characters. The image-stacking technique was not always applicable because in some cases the stacking of images added too much noise or artefacts to the final composition. In such cases we opted to show different focal planes of the same structure side by side (*e.g.* Fig. 5).

The macro-specimens and thin sections are housed at the Museo Regional Padre Molina of Río Gallegos (Santa Cruz Province, Argentina) under accession numbers MPM-Pb-16033;16039-16045.

RESULTS

Description

Fungal specimens occur in association with root nodules that emerge from conifer fine roots. These are preserved in chert and obliquely-cross and longitudinally sectioned (Fig. 2a). A central vascular cylinder with secondary xylem followed by the cortex and an apparent periderm are observed in oblique-cross section of older roots (Fig. 2b). In oblique-longitudinal section, scarce diagnostic characters of the secondary xylem are observed (Fig 2c-e). Pits in the radial walls of the tracheids are contiguous, biseriate or uniseriate with occasional biseriate rows (Fig. 2d-e).

The young fine nodular roots are 0.5 to 1 mm in diameter. Some roots are constrained, showing a beaded appearance (Fig. 3a). The nodules emerge at right angle from the parental root (Fig. 3c) and are arranged in variable patterns (Fig 2f), some are densely packed, others scattered, and in some specimens they are alternately arranged (Fig 3a). They are spherical to spheroidal (*i.e.* longitudinally elongated) (Fig. 2a,f; Fig. 3a-e) and measure 0.8 (0.45-1.18) mm in diameter.

Anatomically, the nodules are characterized by a central vascular cylinder of 92 (78-120) µm of diameter surrounded by an endodermis of 24 (11-35) µm wide.

The endodermis is sorrounded by the cortex, ending in an epidermis, and the cortex is 340 (250-445) µm wide (Fig. 3c-d). Root hairs or their bases are occasionally observed (Fig. 3d,f). Root hairs are approximately 19.5 µm in diameter, they are wider at the base with an approximate diameter of 25 µm.

The nodules originate at the protoxylem poles of the roots (Fig. 3b). The vascular strand and the endodermis can be followed from the main root up to the middle portion of the nodule in longitudinal section (Fig. 2f; 3a-e). Cortical tissues are composed of 6-8 (10) layers of cortical cells, cells get flattened towards the epidermis (Fig. 3d). Resurgent growth can be observed in some nodules, where new cortical tissue grows inside the older cortex (Fig. 3e).

The cortex of the root nodules is colonized by arbuscular mycorrhizal fungi (Fig. 4, 5, 6, 7). Additionally, AM fungal structures occur in the chert matrix, very close or directly attached to the roots and nodules (Fig. 7). The fungi are mainly restricted to the inner layers of cortical tissue whereas cortical layers below the epidermis remain almost fungal free. In the inner zone of the cortex, the outer layers are occupied by intra-cellular coenocytic hyphae that sometimes form coils (Fig. 4a-f). The hyphae are irregular in diameter, ranging from 3,4 to 14,5 µm, the average diameter is 7 µm. The hyphae also develop knobs along its length (Fig. 4b), branch dichotomously (Fig. 4c) and at right angle (Fig. 4d). Some coils display possible circular to ellipsoidal scars and are directly associated to narrower hyphae of an intrusive septate fungus (Fig. 4e-f). However, this scars can be preservation artefacts, rendering the metabolic status of the septate fungus saprothrophic or parasitic.

Towards the inner cortex, the hyphae becomes thinner, developing highly ramified structures that resemble arbuscules (Fig. 5a,c). Arbuscules are observed partially occupying the cells lumens (Fig. 5c; 6a-f; see also supplementary material). Trunk hyphae of the arbuscules are less than 4 µm in diameter.

Morphologically identical, intra- and extra-rhizal glomoid spores are also observed (Fig. 7a-f). Intra-rhizal spores occur in the cortex of both, young roots and nodules. Extra-rhizal spores are observed dispersed in the chert matrix very close to the roots and nodules. Extra-rhizal mycelium associated to extra-rhizal spores (Fig. 7b,c) extends parallel to the roots and nodule's surface. The hyphae measure 1.5 to 4.5 µm in diameter and present Y-branching. In some specimens, coenocytic hyphae emerging from the spores attached to the epidermal surface of nodules (Fig. 7b-c) and possibly penetrating the epidermal cells are observed (Fig. 7d).

The spores are terminal, globose to subglobse, variable in size, ranging from 26.5 to 43.5 µm in diameter (Fig. 7e-f). The spore wall appears to be comprised of two layers (Fig. 7e-f). The outer layer is hyaline and the inner layer is opaque. The spores are occluded at their attachment point by a fold of the inner layer in some specimens (Fig. 7f), or display a plug in others (Fig. 7e). The spores are attached to simple subtending hyphae. The hyphae are coenocytic and 2.0 (1.2-2.9) µm in diameter.

DISCUSSION

Here we described an ancient plant-fungal association in the root nodules of Jurassic conifers. Coenocytic hyphae forming coils, ramified arbuscules, and spores, attributable to glomeromycotan fungi occur inside the cortical tissues of

229 conifer root nodules. We observe that the AM fungal structures are restricted to the
230 inner layers of the cortex, whereas only a few scattered coenocytic hyphae occur in
231 the outer cortex, close to the entry points below the epidermis. When resurgent
232 growth in the host nodules is present, hyphae are also present in the renewed
233 cortex of the nodules, which positively compares to what has been described in
234 extant Araucariales (Bailys et al. 1963, Russell et al. 2002; Dickie and Holdaway
235 2011). Analogous general distribution of AM fungi in host nodules is also observed
236 in extant Araucariales (Fig. 5b; Table 1; McGee et al. 1999; Russell et al. 2002;
237 Dickie and Holdaway 2011). Similarly, previous records of fossil mycorrhizal plants
238 found identically zoned distribution of AM fungi in the infected root tissues (Remy
239 et al. 1994; Phipps and Taylor 1996; Strullu-Derrien et al. 2009; Strullu-Derrien et
240 al. 2014; Harper et al. 2015).

241 Two morphological types of arbuscular mycorrhizae have been classically
242 distinguished (Gallaud 1905): *Arum* type, which includes intercellularly hyphal
243 growth in a longitudinal pattern in the cortex, with short side branches that give rise
244 to arbuscules nearly at right angle; and *Paris* type, characterized by intracellular
245 coiling hyphae with arbuscules forming along the coil (Taylor et al. 2015; Walker et
246 al. 2018). Features of the fungi described here are consistent with the *Paris*
247 morphological type. However, it has been shown that the morphological types vary
248 in a continnum depending on a series of factors (e.g. host plant tissues, taxa
249 involved; environment; Dickson 2004; Van Aarle et al. 2004; Kubota et al. 2005),
250 rendering this character merely descriptive and of relative use for the classification
251 of AM fungi. Besides, the distinction of these morphologies have been used in
252 fossil comparisons (Harper et al. 2015; Harper et al. 2016; Walker et al. 2018).

253

254 *Systematic affinities of the root nodules and the fungi*

255 The root nodules described here are characterized by a central vascular
256 cylinder surrounded by an endodermis, followed by several layers of cortex
257 surrounded by an epidermis; spherical to spheroidal three dimensional
258 morphology; and emergence at right angle from the parental root in variable
259 patterns along the main axis. These are features shared by extant Araucariales,
260 which includes the Podocarpaceae and Araucariaceae (see comparisons in Table
261 1). Additional vegetative and reproductive organs assignable to Araucariaceae and
262 Podocarpaceae are found in the La Matilde Formation (e.g., wood, leafy branches,
263 cones; Gnaedinger 2007; Zamuner and Falaschi 2005; Falaschi et al. 2011;
264 Kloster and Gnaedinger 2018; Escapa et al. in prep). Further refinement of the
265 systematic affinities of the nodules based on the morphological characters
266 preserved, especially considering morphological variability known to be present in
267 fossil and extant Podocarpaceae and Araucariaceae, is not possible (Spratt 1912;
268 Dickie and Holdaway 2011; Tables 1,2). Considering the presence of both
269 podocarpaceous and araucariaceous organs in the chert blocks studied and the
270 little morphological variation observed among the mycorrhizal nodules it remains
271 possible that more than one taxa is present.

272 Based on phylogenetic analysis of combined molecular and morphological
273 data, several families have been recognized among the former Glomeromycota
274 (Schüβler et al. 2001; Spatafora et al. 2016): Paraglomeraceae,
275 Archaeosporaceae, Geosiphonaceae, Ambisporaceae, Glomeraceae,
276 Claroideoglomeraceae, Pacisporaceae, Gigasporaceae, Pacisporaceae,

277 Sacculospporaceae, Acaulosporaceae and Diversisporaceae (see Schüβler and
278 Walker 2010; Redecker et al. 2013; http://www.amf-phylogeny.com). Differences
279 mainly in spore type formation, spore wall structure, presence of vesicles, and
280 even ecology, exclude affinities of the fungi described here with several different
281 groups (see Table 3). Particular morphological characters suggest that these
282 fossils most probably affinities are with the Paraglomeraceae. Diagnostic
283 characters supporting affinities with the Paraglomeraceae include arbuscules
284 attached to narrow trunks (<4 µm in diameter), development of intra-rhizal spores
285 and intracellular hyphae frequently coiling. However, *Paraglomus* spores have a
286 three-layered wall, whereas in our specimens, the wall is comprised by two layers.
287 Nonetheless, the outer wall of the spores in *Paraglomus* has been mentioned to
288 disintegrate at senescence, resulting in a two-layerd spore wall and thus,
289 comparable to that displayed by the fossils described here (Morton and Redecker
290 2001). Alternatively, affinities of the fossil with Glomeraceae are also possible,
291 being different mainly based on the absence of vesicles; additionally, the
292 Glomeraceae commonly develop straight hyphae, whereas, the fossil most
293 frequently shows coiled hyphae. These same features also make the fossil
294 somewhat comparable to Diversisporaceae and Ambisporaceae, although these
295 two families develop acaulosporoid spores, which have not been observed in the
296 fossil.
297
298 *Fossil conifers with arbuscular mycorrhizal fungi*
299 The oldest record of nodular roots colonized by arbuscular mycorrhizal fungi
300 is from the Triassic of Antarctica (Schwendemann et al. 2011; Harper et al. 2015).

In this case, both the rootlets and the root nodules of *Notophytum krauselli* are colonized by serveral fungal estructures (i.e. hyphal coils, arbuscules, extra-rhizal hyphae, vesicles and spores; Schwendemann et al. 2011; Harper et al., 2015). However, fungal structures found in the nodules are different from those found in the secondary rootlets, suggesting different arbuscular mycorrhizal symbiosis occurring in different organs of the same plant root system (Harper et al. 2015). Other examples of conifers showing root nodules are from different Cretaceous localities from the Southern Hemisphere (Cantrill and Douglas 1988; Cantrill and Falcon-Lang 2001; Banerji and Ghosh 2002). The lack of anatomical preservation in these nodules found as impressions, compressions and molds prevents any fungal association to be observed. Numerous affinities were discussed for these Cretaceous conifer nodule-bearing roots based on the association with other organs, including affinities with the Podocarpaceae, Araucariaceae, Sciadopityaceae, and even Cupressaceae. This new record of arbuscular mycorrhizal in root nodules with affinities with the Araucariales from the Jurassic of Patagonia fills a gap in the geological history of AM fungi and further documents morphological diversity and ecological preferences of glomeromycetes through time.

Mycorrhizal nodules and ecology

The exceptional preservation of the conifer nodular roots of the 'Cañadón Nahuel' locality can be related to the mode of preservation typical of hot-spring settings, where plants and other organisms become immersed in a mineral-saturated solution that gradually transform them into fossils (Channing and

Edwards 2004; Hellawell et al. 2015). The presence of fungal structures inside the cortex of these underground organs indicates that the fossilization process of these organisms must have occurred while they were alive or soon after their death (Channing and Edwards, 2004). Such fossilization process consisted in permineralization by silica, which is a characteristic previously observed in 'La Bajada' and 'San Agustín' hot-spring localities also of the La Matilde Formation (García Massini et al. 2016). Moreover, based on their distribution and textural and fossiliferous content, the plant-rich cherts of the 'Cañadón Nahuel' locality are related to the distal areas of siliceous sinter geothermal system, potentially analogue to modern Yellowstone National Park geothermally influenced environments in the USA (Channing et al 2009a,b). Root nodules have also been preliminary described in the above mentioned 'San Agustín' and 'La Bajada' localities (see supplemental material; García Massini et al., 2013, 2016). These root nodules share the general morphology and anatomy of the specimens described here, though the best preserved examples of arbuscular mycorrhizal colonization are those from 'Cañadon Nahuel'.

Presence of arbuscular mycorrhizal fungi is not needed for the development of the root nodules in extant Araucariales, as they are indeed part of the root system ontogeny (Baylis et al. 1963; Baylis 1969), however, these organs are hypothesized to increase the volume of fungal colonization at a lower energy cost than having an extensive root system and therefore maximize mineral nutrient acquisition efficiency (Russell et al. 2002; Dickie and Holdaway 2011). Although the role of the mycorrhizal nodules in the extant Araucariales is not yet completely understood, these organs are thought to be ecologically significant with respect to

uptake of water and nutrients, especially phosphorous and nitrogen; additionally, establishment of seedlings in nutrient-poor environments has also been suggested to be influenced by the development of mycorrhizal nodules (Russel et al. 2002; Padamsee et al. 2016; Dickie and Holdaway 2011 and citations therein). Systematic and taxonomical refinement to the family level of the conifers of 'Cañadón Nahuel' and further characterization of the paleo-environments where this plants used to live should enrich what can be grasped from these fossils on the evolution and significance of the AM symbiosis in the Jurassic Araucariales of Patagonia.

CONCLUSIONS

We present the first record of structurally preserved arbuscular mycorrhizal fungi colonizing root nodules from the Jurassic, partially filling a gap in the Mesozoic record of conifer mycorrhizal nodules, and extending the record geographically.

The mycorrhizal nature of the fungus is demonstrated by the presence of several fungal structures in a delimited zone of the nodule's cortex, especially important is the presence of hyphal coils, arbuscules and glomoid spores.

The nodules have affinities with the Araucariales clade of conifers, however, it is not possible to constrain the affinities of these organs further based on the anatomical evidence preserved. Nonetheless, the presence of these structures is important as they expand our knowledge on the morphology and ecology of these conifers during the establishment of the modern conifer families in the Jurassic. They also open the possibility to work on whole-plant concept reconstructions in

373 the future.

374

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591 TABLES AND FIGURES CAPTIONS

592

593 Table 1. Comparisons of 'Cañadón Nahuel' locality specimens of root nodules and
594 the root nodules of extant Podocarpaceae and Araucariaceae species.
595 AMF = arbuscular mycorrhizal fungi; PS = parallel series; LS = longitudinal series;
596 VC = vascular cylinder; MR = main root; En. = endodermis; C. = cortex; E. =
597 epidermis; Ex = exodermis; VS = vascular strand; P = Present; A = absent; Sc. =
598 Scattered; ? = Missing information
599 Information taken from:
600 *a Breuniger et al 2000
601 *b McGee 1990
602 *c Godoy and Mayr 1989
603 *d Padamsee et al 2000
604 *e Russel et al 2002; Dickie and Holdaway 2011
605 *f Leone et al 2008

606

607 Table 2. Fossil record of conifer root nodules. Modified from Schwendemann et al.

608 2011.

609 AMF = arbuscular mycorrhizal fungi; P = preserved; NP = not preserved; ? =

610 missing information

611

612 Table 3. Comparisons within families of the former Glomeromycota that form

613 glomoid spores.

614 G = glomoid; Ac. = acaulosporoid; Occ. = occlusion; P = present; A = absent; ? =

615 missing information or not clear

616

617 *a Schußler and Walker 2010

618 *b Ohel and Sieverding 2004

619 *c Schußler 2002

620 *d Walker et al 2007

621 *e Walker and Schußler 2004

622 *f Morton and Benny 1990

623 *g Morton and Redecker 2001

624 *h Redecker et al 2013

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626 FIGURE 2. Thin sections of chert blocks from 'Cañadón Nahuel' locality, MPM-Pb-

627 16039-16041. – a. General view of chert block showing conifer roots immersed in

628 the matrix. Some nodules in young fine are colored with pink sof-light. – b.

629 Oblique-cross section of conifer diarch root. Note secondary xylem (2ºX), cortex

(C) and apparent periderm (Pd). – c. Oblique-longitudinal section of conifer root showing secondary xylem (2ºX) and cortex (C), periderm not observed. – d and – e. Detail of secondary xylem. Note araucarian pitting in the walls of the tracheids. – f. General view of nodular root with spherical to spheroidal nodules, disposed with any apparent pattern. Scale bar for b and c = 0.5 mm; for d and e = 10 µm; for f = 100 µm.

FIGURE 3. Thin sections of chert blocks from 'Cañadón Nahuel' locality, MPM-Pb-16033;16039-16040. – a. General view of 'beaded' root with nodules, nodules disposed alternately. – b. Cross section of a nodule, showing vascular trace (VT) emerging from the parental root. – c. Oblique-longitudinal section of root, with lateral root nodule emerging at right angle from the main axis. – d. Cross section of a nodule surrounded by the main root cortex, showing general anatomy of the nodule. Note vascular strand (VS), endodermis (En), Cortex (C) and epidermis (E). Detail of vascular strand and endodermis inside box, scale bar = 20 µm. – e. Detail of root nodule showing resurgent growth, new cortex (NC) developing inside the cortex of the older nodule (OC). – f. General view of root hairs. Scale bar for a. 0.5 mm; for b, c, d, e and f = 100 µm.

FIGURE 4. Thin sections of chert blocks from 'Cañadón Nahuel' locality, MPM-Pb-16040-16041. – a. General view of root nodule. Pink coloration indicates the presence of hyphal coils. – b. Detail of a coenocytic hyphal coil showing knobs. – c. Detail of coenocytic hyphae showing Y-branching. – d. Detail of coenocytic hyphae showing branching at right angle. – e and f. Detail of possible worn out hyphae

intruded by a septate fungus (white arrow heads). Scale bar for a = 100 µm; for b, c, d, e and f = 10 µm.

FIGURE 5. Thin section of chert block from 'Cañadón Nahuel' locality, MPM-Pb-16039, and thin section of extant Podocarpaceae *Podocarpus totara* root nodule (Images by Larry Peterson and Lewis Melville, from Dickie & Holdaway, 2011) – a. Longitudinal section of fossil root nodule showing zone of the cortex occupied by arbuscules (delimited by dotted line) – b. Longitudinal section of extant root nodule showing zone of the cortex occupied by arbuscules (delimited by dotted line). – c. Detail of arbuscules in fossil root nodule. – d. Detail of arbuscules in extant root nodule. Scale bar for a and b = 100 µm; for c = 20 micras; for d = 50 µm

FIGURE 6. Thin section of chert block from 'Cañadón Nahuel' locality, MPM-Pb-16039. Diversity of arbuscules partially occupying cortical cells of root nodules. Scale bar for all = 10 µm.

FIGURE 7. Thin section of chert block from 'Cañadón Nahuel' locality, MPM-Pb-16039. – a. Root nodule with intra-rhizal glomoid spores. – b. Glomoid spore associated to root nodule. Note connecting hypha (arrowhead). – c. Glomoid spore with multiple hyphal attachments, some of which connect it to the root nodule. – d. Magnification of previous image showing detail of entry point with penetrating hyphae and development of coil. – e. Detail of intra-rhizal globose spore in decaying root, showing two-layered wall, simple subtending hypha and occlusion

677 plug. – d. Detail of extra-rhizal globose spore, showing two-layered wall, simple
678 subtending hypha, and occlusion.
679
680 SUPPLEMENTAL MATERIAL
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682 Appendix 1. – A1. Diversity of young fine roots and nodules from different localities
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689
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Conifer root nodules ~~nodules~~ colonized ~~with~~ by arbuscular ~~mycorrhizae~~ mycorrhizal fungi in Jurassic geothermal settings from Patagonia, Argentina

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ABSTRACT

Premise of the Research: Despite their ecological significance in modern terrestrial ecosystems, knowledge about the evolution of arbuscular mycorrhizae based on the fossil record is still scarce, ~~including very few examples of mycorrhization in conifers~~especially concerning the case of root nodules harboring arbuscular mycorrhizal fungi as in some extant gymnosperms and angiosperms. Exceptionally preserved conifer nodular roots were found in the Jurassic fossil-bearing chert deposits of the Deseado Massif (Santa Cruz, Argentina), raising the

possibility to study them in association with arbuscular mycorrhizal fungi. The aim of this study is to describe the plant organs and their fungal partners, and to discuss the ecological significance of the interactions observed, particularly with respect to their occurrence in the hot-spring settings.

Methodology: Thin sections of chert samples from the 'Cañadón Nahuel' locality of the La Matilde Formation, Deseado Massif (Santa Cruz, Argentina) were observed using light microscopy.

Pivotal Results: The cortex of the nodules ~~and nodule-bearing roots~~ are occupied by several glomeromycotan fungal structures. The structures occur in a specific zone of the cortex - towards its center -, and includes intracellular hyphal coils and arbuscules. Glomoid spores, and ~~penetrating~~ coenocytic hyphae possibly ~~at entry points~~penetrating the epidermal cells are also described and analyzed.

Conclusions: The root nodules have affinities with the Araucariales, ~~comprising~~ representing the oldest record of such structures for this conifer clade. This is also the first record ~~of mycorrhizal nodules~~nodules harboring arbuscular mycorrhizal fungi for the Jurassic~~, ; and one of the very few examples of structurally preserved mycorrhizae in conifers~~it extends our knowledge of the fossil record of this particular type of fungal association. ~~The presence of this symbiosis may have influenced the distribution of both, plants and fungi in the distal areas of the hot-spring influenced environments of Patagonia.~~

Keywords

Root nodules; arbuscular ~~mycorrhizae~~mycorrhizal fungi; hot-spring settings; Araucariales; Jurassic

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51 INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) have a deep fossil record (Pirozynski and Dalpé 1989; Remy et al. 1994; Taylor et al. 2015; Walker et al. 2018 and citations therein) and today develop symbiosis with approximately 80% of vascular plants worldwide, including five major groups of living conifers (Brundrett 2004; Smith and Read 2008; Spatafora et al. 2016; Wang and Qiu 2016; Brundrett and Tedersoo 2018). In modern conifers, AMF colonize roots of the Taxaceae, Cupressaceae, Sciadopityaceae, Araucariaceae and Podocarpaceae (Brundrett 2009; Wang and Qiu 2006; Dickie and Holdaway 2000). The members of the Araucariales clade (*i.e.* members of the Podocarpaceae and Araucariaceae) and *Sciadopytis* harbor the AMF in young fine roots and small spherical differentiated structures called nodules (Spratt 1912; Godoy and Mayr 1989; Breuninger et al. 1999; Mcgee et al. 1999; Russell et al. 2002; Leone et al. 2008; Dickie and Holdaway 2011; Padamsee et al. 2016). Although AMF are relatively well-known from the fossil record (Taylor et al. 2015; Strullu-Derrien et al. 2018; Walker et al. 2018), the fossil record of arbuscular mycorrhizal associations with conifers and related groups is sparse.

Structurally preserved ~~Fungi are important in modern terrestrial ecosystems as they establish a broad spectrum of mutualistic to parasitic symbiotic interactions with many organisms, including plants (Blackwell 2011, Taylor et al. 2015 and citations therein). Mycorrhizal symbiosis is perhaps the most important plant-fungal interaction developed, occurring on approximately 85% of the living plants and involving several clades of fungi (Brundrett 2004; Wang and Qiu 2006; Smith and Read 2008; Brundrett and Tedersoo 2018). This mutualistic interaction results in nutritional benefits for both partners involved, and confer other benefits to host plants, such as stress tolerance and protection against pathogens (Marx 1972; Newsham et al. 1995; Read 1999; Borowicz 2001; Bunn and Zabinski 2003; Hildebrant et al. 2007; Miransari 2010).~~

~~The most widespread and ecologically important type of mycorrhiza are the arbuscular mycorrhizae (AM), also referred to as endomycorrhizae (Smith and Read 2010). Arbuscular mycorrhizal symbiosis occur between fungi in the former Glomeromycota (Schüßler et al. 2001), now the Glomeromycotina and Mucoromycotina groups (Spatafora et al. 2016), and the roots of the majority of vascular plants (Schüßler et al. 2001, Strullu-Derrien and Strullu 2007; Strullu-Derrien et al. 2018; Taylor et al. 2015; Walker et al. 2018; Brundrett and Tedersoo 2018). Arbuscular mycorrhizae play an important role in the uptake of water and soil nutrients, specially phosphorus and nitrogen (Augé 2001; Smith and Read 2010; Smith and Smith 2011; Wipf et al. 2019). For this, the AM fungi display an extensive hyphal network in the soil, considerably expanding the absorption function carried out by roots (Taylor et al. 2015; Redecker and Schüßler 2014).~~

Inside roots the exchange between the host plant and the fungus is enhanced by the development of highly ramified hyphal structures called arbuscules (Smith and Read 2010; Redecker and Schüßler 2014, Walker et al. 2018).

Both the fossil record and the molecular analyses suggest that AM symbiosis are the ancestral type and have driven colonization of land by plants (Pirozynski and Malloch 1975; Wang et al. 2010; Redecker et al. 2000; Schüßler et al. 2001; Wang and Qiu 2006). From early land plants to modern trachaeophytes, glomeromycotan fungi forming AM and similar symbiosis are present in several lineages of extinct and extant plants, including bryophytes, rhyniophytes, lycopsids, ferns, pteridosperms, cycads, ginkgoales, conifers and angiosperms (Strullu-Derrien and Strullu 2007; Strullu-Derrien et al. 2018; Brundrett and Tedersoo 2018, and citations therein).

In modern conifers, arbuscular mycorrhizae are present in the Taxaceae, Cupressaceae, Sciadopityaceae, Araucariaceae and Podocarpaceae, whereas Pinaceae typically develop ectomycorrhizae (Brundrett 2009; Wang and Qiu 2006; Dickie and Holdaway 2000; Brundrett and Tedersoo 2018). The members of the Araucariales clade (*i.e.* members of the Podocarpaceae and Araucariaceae) and *Sciadopytis* harbour the AM fungi in young fine roots and small spherical differentiated structures called nodules (Spratt 1912; Godoy and Mayr 1989; Breuninger et al. 1999; Mcgee et al. 1999; Russell et al. 2002; Leone et al. 2008; Dickie and Holdaway 2011; Padamsee et al. 2016). They are suggested to increase the root colonization by AM fungi at a lower cost than developing an extensive root system (Mcgee et al. 1999; Dickie and Holdaway 2001).

~~Preservation of fungi in their original context and three-dimensionally provide a unique way to study their evolution and significance in past terrestrial ecosystems (Taylor and Taylor 2000; Taylor et al. 2015, García Massini et al. 2012). Despite the importance of mycorrhizae, however, the fossil record of these plant-fungal interactions is still scarce, including very few examples of mycorrhizal conifers (Strullu-Derrien and Strullu 2007; Strullu-Derrien et al. 2018; Walker et al. 2018, and citations therein). The oldest fossil record of~~ AM~~F~~ have been described in Carboniferous Cordaites from the Massif Central, France (Strullu Derrien et al. 2009), ~~in in~~conifers ~~is comprised by~~ voltzialean conifers from the Triassic of Antarctica (Schwendemann et al. 2011; Harper et al. 2015~~);~~), and in ~~further AM symbiosis was inferred in roots related the Araucariales are known from the Cretaceous of Antarctica, Australia and India (Cantrill and Douglas 1988; Cantrill and Falcon-Lang 2001; Banerji and Ghosh 2002); AM fungi were also described from~~ a taxodiaceous Cupressaceae from the Middle Eocene of Canada (Stockey et al. 2001). Nodular roots related to the Araucariales are known from the Cretaceous of Antarctica, Australia and India (Cantrill and Douglas 1988; Cantrill and Falcon-Lang 2001; Banerji and Ghosh 2002), and in the ~~Regarding mycorrhizal root nodules, the only structurally preserved examples are those of the~~ Triassic voltzialean *Notophytum krauselli* (Schwendemann et al. 2011, Harper et al. 2015). This last record is the only one showing anatomical preservation of the rootlets in which AM fungal colonization has been described.

The Middle-Late Jurassic chert deposits of the Bahía Laura Group in the Deseado Massif of Santa Cruz Province, Southern Patagonia, Argentina bear exceptionally preserved organisms in hot-spring settings (Channing et al. 2007;

Guido et al. 2010; Channing et al. 2011; García Massini et al. 2012, 2016). The best preserved Jurassic geothermal paleosurfaces can be positively compared based on their structure to hot springs at Yellowstone National Park in the United States (Channing et al. 2009a,b). These are the only geothermal fossiliferous deposits with an ecosystem-level preservation known for the Mesozoic. Preliminary reports on the fossiliferous content and paleoenvironment reconstruction have been carried out on a few localities (Channing et al. 2007; Guido et al. 2010; García Massini et al. 2012; García Massini et al. 2016; Sagasti et al. 2016). Plant remains ~~are abundant in these localities, including completely preserved horsetails (Channing et al. 2011; García Massini et al. 2016), and diverse organs of gleicheniaceous and osmundaceous ferns, cycads, and conifers (Guido et al. 2010; Sagasti et al. 2016; García Massini et al. 2016). Among conifer remains, several dispersed organs were identified including wood fragments, leafy twigs, isolated single and multi-veined leaves, isolated ovules, pollen cones, pollen grains in the interior of cones and dispersed in the chert matrix, and roots. All these organs have suggested affinities with the Araucariaceae and Cheirolepidiaceae (Guido et al. 2010; García Massini et al. 2016; Sagasti et al. 2018). Affinities with the Podocarpaceae family are discussed for particular brachyphyllous stems bearing single veined leaves and pollen cones with *in situ* bisaccate pollen, by Escapa et al. (in prep.). Animal remains (*e.g.* arthropods, mollusks), algae, bacteria, protists, coprolites and traces have also been preliminarily described (Guido et al. 2010; García Massini et al. 2016).~~and ~~Fungal~~ fungal and fungus-like vegetative and reproductive structures are ~~also~~ abundant (Guido et al. 2010, García Massini et al. 2012, García Massini et al. 2013; García Massini et al. 2016,

Sagasti et al. 2016, Sagasti et al. 2019), and ~~these are~~ frequently in ~~associated~~ association with each other ~~with different plant remains~~ (*e.g.* García Massini et al. 2016). This provides the opportunity to describe a variety of plant-fungal interactions to ~~further~~ characterize the dynamics of the hot-springs associated ecosystems and to broaden our knowledge of fungal evolution and diversity during the Jurassic, and in a broader sense during the Mesozoic.

In this contribution we describe particular conifer nodular ~~fine~~ roots colonized by AM fungi, ~~present~~ preserved as inclusions in the matrix ~~in~~ of chert blocks collected from the recently discovered 'Cañadón Nahuel' locality of the La Matilde Formation, Bahía Laura Group, at the Deseado Massif hot-spring deposits. We discuss possible systematic affinities for these roots based on comparisons with extinct and extant examples of conifer nodular roots. We also address the ecological significance of the arbuscular mycorrizhal interaction between the studied conifers and glomeromycotan fungi in the context of the hot-springs settings. This finding expands our knowledge on fungal diversity and ecology in the Jurassic and fills a gap in the fossil record of arbuscular mycorrhizal symbiosis in conifers.~~.~~

MATERIALS AND METHODS

Geological setting

The studied samples were collected at the 'Cañadón Nahuel' locality of the La Matilde Formation, in the Bahía Laura Complex of the Deseado Massif, Santa Cruz Province, Argentina (Fig. 1). The Deseado Massif is an extensive (60,000 km^2) region in southern Patagonia, in Santa Cruz Province, Argentina. The geologic

history of this regiǫ́n is marked by volcanic activity during the Jurassic. Approximately 178-151 million years ago (Pankhurst et al. 2000), this area was subject to bimodal rhyolitic and andesitic volcanism, which lead to the formation of the Bahía Laura Complex (Guido 2004). Rocks of the Bahía Laura Complex are part of the Chon Aike Silicic Large Igneous Province (which ranges from Patagonia to Antarctica; Pankhurst et al. 1998). This province's genesis is related to ~~crustal thinning in a diffuse extensional back-arc setting associated with~~ the break-up of Gondwana and birth of the South Atlantic Ocean (Riley et al. 2001; Richardson and Underhill 2002).

Widespread hydrothermal activity in the Deseado Massif resulted from the mature (quiescent) volcanic phase in this region during the Late Jurassic, which produced metalliferous epithermal deposits (i.e. formed at shallow depths related to the terrestrial surface, in the upper portion of a hydrothermal system under temperatures between 150 and 300 ºC), extensive silicification, and formation of geothermal fields at the surface (Schalamuk et al. 1997; Guido and Campbell 2011). Well-preserved fossilized geothermal fields have been found within volcaniclastic fluviolacustrine strata, distributed in a 400 x 250 km area of NNW-SSE and WNW-ESE oriented belts aligned with major regional structures (Guido and Campbell 2011). Twelve of these geothermal fields preserve fossil assemblages, which represent the only known fossiliferous Mesozoic hot-springs. These deposits fill a significant stratigraphic gap between similar Paleozoic and Cenozoic deposits (Channing and Edwards 2013).

'Cañadón Nahuel' (GPS coordinates available upon request) is the farthest east of these chert deposits in the Deseado Massif (Fig. 1). The locality is located

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in the Northern Belt defined by Guido and Campbell (2011). Cherts from 'Cañadón Nahuel' are rather randomly distributed over a large area and show textural and fossiliferous characteristics that relate them to formation in the distal parts or fringes of a siliceous sinter geothermal system. The observed distribution of the cherts suggests they have been successively removed by ~~is interpreted as having resulted from explosive~~ recurrent volcanic and phreatic local eruptions or breccias related to the geothermal activity~~that successively removed cherts formed by dynamic geothermal activity~~. In some instances, horizontal bed cherts of variable extension are found and these too are interpreted as having formed in wetlands and shallow saline ponds, in the distal parts of geothermal systems.

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Preparation and imaging of studied materials

Thin sections of chert blocks were prepared following standard methodology (Jones and Rowe 1999). Slides were observed using transmitted light microscopy. All images were taken with a Nikon DS-Fi1 camera connected to a Nikon Eclipse 80i microscope. General views of the sections were taken using a Canon 7D camera with a Canon macro lens of 60 mm, in a light box with fluorescent tubes. At higher magnifications, multiple images of the same structure of interest were taken at different focal planes to compose an image with greater depth of focus, following an image-stacking technique (Bercovici et al. 2009). We used Helicon Focus software (http://www.heliconsoft.com/heliconfocus.html), with the ''Method B'' (Depth Map) parameter. Several partially overlapping images were merged to create high-quality images of critical features. This technique was applied using Adobe Photoshop CS6, and images were processed after to reveal critical

characters. The image-stacking technique was not always applicable because in some cases the stacking of images added too much noise or artefacts to the final composition. In such cases we opted for showingto show different focal planes of the same structure side by side (*e.g.* Fig. 5).

The macro-specimens and thin sections are housed at the Museo Regional Padre Molina of Río Gallegos (Santa Cruz Province, Argentina) under accession numbers MPM-Pb-16033;16039-16045.

RESULTS

Description

Fungal specimens occur in association with root nodules that emerge from conifer fine roots. These are preserved in chert and obliquely cross and longitudinally sectioned (Fig. 2a). A Oblique cross and longitudinal sections of anatomically preserved conifer diarch roots with secondary growth and young fine roots bearing nodules immersed in siliceous chert matrix are described (Fig. 2a).

A central vascular cylinder with secondary xylem followed by the cortex and an apparent periderm are observed in oblique-cross section of older roots (Fig. 2b). In oblique-longitudinal section, scarce diagnostic characters of the secondary xylem are observed (Fig 2c-e). Pits in the radial walls of the tracheids are contiguous, biseriate or uniseriate with occasional biseriate rows. (Araucarian pattern *sensu* Phillipe and Bamford (2008)) (Fig. 2d-e).

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The young fine nodular roots are 0.5 to 1 mm in diameter. Some roots are constrained, showing a beaded appearance (Fig. 3a). The nodules emerge at right angle from the parental root (Fig. 3c) and are ~~disposed~~ arranged in variable patterns (Fig 2f), some are densely packed, others scattered, and in some specimens they are alternately arranged (Fig 3a). They are spherical to spheroidal (*i.e.* longitudinally elongated)~~,~~ (Fig. 2a,f; Fig. 3a-e) and measure 0.8 (0.45-1.18) mm in diameter.

Anatomically, the nodules are characterized by a central vascular cylinder of 92 (78-120) µm of diameter surrounded by an endodermis~~-~~ of 24 (11-35)~~that is seven times thinner than the vascular cylinder~~ µm wide.~~.~~ The endodermis is ~~followed~~ surrounded by the cortex, ending in an epidermis, and the cortex is ~~is four times as wide as the vascular cylinder~~340 (250-445) µm wide (Fig. 3c-d). Root hairs or their bases are occasionally observed (Fig. 3d,f). Root hairs are approximately 19.~~,~~5 µm in diameter, they are wider at the base with an approximate diameter of 25 µm.

The nodules originate at the protoxylem poles of the roots (Fig. 3b). The vascular strand and the endodermis can be followed from the main root~~, generally traversing~~up to the middle portion of the nodule in longitudinal section (Fig. 2f; 3a-e). ~~Cortex~~ Cortical tissues are~~is~~ composed of 6-8 (10) layers of cortical cells, cells get flattened towards the epidermis (Fig. 3d). Resurgent growth can be observed in some nodules, where new cortical tissue grows inside the older cortex (Fig. 3e).

The cortex of ~~fine roots and~~the root nodules are colonized by arbuscular mycorrhizal fungi (Fig. 4, 5, 6, 7). Additionally, AM fungal structures occur in the

chert matrix, very close or directly attached to the roots and nodules (Fig. 7). The fungi are mainly restricted to the inner layers of cortical tissue whereas cortical layers below the epidermis remain almost fungal free.

~~The outer layers of cortical tissue, below the epidermis, are mainly devoid of fungal structures. The majority of the fungal structures are restricted to the inner layers of the cortex.~~ In the ~~this specific~~inner zone of the cortex, the outer layers are occupied by intra-cellular coenocytic hyphae that sometimes form coils (Fig. 4a-f). The hyphae are irregular in diameter, ranging from 3,4 to 14,5 µm, the average diameter is 7 µm. The hyphae also develop knobs along its length (Fig. 4b), branch dichotomously (Fig. 4c) and at right angle (Fig. 4d) ~~and present dichotomous Y-branching (Fig. 4c)~~. Some coils ~~present~~ display possible circular to ellipsoidal scars and ~~appear to be attacked by~~are directly associated to narrower hyphaer of an intrusive septate fungus (Fig. 4e-f). However, this scars can be preservation artefacts, rendering the metabolic status of the septate fungus saprothrophic or parasitic. ~~(Fig. 4e-f).~~

Towards the inner cortex, the hyphae becomes thinner, developing highly ramified structures that resemble arbuscules (Fig. 5a,c). Arbuscules are observed partially occupying the cells lumens~~, and traverse laterally from a cell to the other~~ (Fig. 5c; 6a-f; see also supplementary material). Trunk hyphae of the arbuscules are less than 4 µm in diameter.

Morphologically identical, intra- and extra-rhizal glomoid spores are also observed (Fig. 7a-f). Intra-rhizal spores occur in the cortex of both, young roots and nodules. Extra-rhizal spores are observed dispersed in the chert matrix very close to the roots and nodules. Extra-rhizal mycelium associated to extra-rhizal spores

(Fig. 7b,c) extends parallel to the roots and nodule's surface. The hyphae measure 1.5 to 4.5 µm in diameter and present Y-branching. In some specimens, coenocytic hyphae emerging from the spores attached to the epidermal surface of nodules (Fig. 7b-c) and possibly penetrating the epidermal cells are observed (Fig. 7d).

The spores are terminal, globose to subglobse, variable in size, ranging from 26.5 to 43.5 µm in diameter (Fig. 7e-f). The spore wall appears to be comprised of two layers (Fig. 7e-f). The outer layer is hyaline and the inner layer is opaque. The spores are occluded at their attachment point by a fold of the inner layer in some specimens (Fig. 7f), or ~~present~~ display a plug in others (Fig. 7e). The spores are attached to simple subtending hyphae. The hyphae are coenocytic and 2.0 (1.2-2.9) µm in diameter.

DISCUSSION

Here we described an ancient plant-fungal association in the root nodules of Jurassic conifers. Coenocytic hyphae forming coils, ~~highly~~ ramified arbuscules, and spores, attributable to glomeromycotan fungi occur inside the cortical tissues~~ex~~ of conifer root nodules. We observe that the AM fungal structures are restricted to the inner layers of the cortex, whereas only a few scattered coenocytic hyphae occur in the outer cortex, close to the entry points below the epidermis. ~~Further, when~~When resurgent growth in the host nodules is present, hyphae are also present in the renewed cortex of the nodules, which positively compares to what has been described in extant Araucariales (Bailys et al. 1963, Russell et al. 2002; Dickie and Holdaway 2011). Analogous general distribution of AM fungi in host nodules is also observed in extant Araucariales (Fig. 5b; Table 1; McGee et al. 1999; Russell et al.

2002; Dickie and Holdaway 2011). Similarly, previous records of fossil mycorrhizal plants found ~~identical~~identically zoned distribution of AM fungi in the infected root tissues (Remy et al. 1994; Phipps and Taylor 1996; Strullu-Derrien et al. 2009; Strullu-Derrien et al. 2014; Harper et al. 2015).

Two morphological types of arbuscular mycorrhizae have been classically distinguished (Gallaud 1905): *Arum* type, which includes intercellularly hyphal growth in a longitudinal pattern in the cortex, with short side branches that give rise to arbuscules nearly at right angle; and *Paris* type, characterized by intracellular coiling hyphae with arbuscules forming along the coil (Taylor et al. 2015; Walker et al. 2018). ~~Coenocytic hyphae are always intracellular and frequently forming coils. Additionally, the coenocytic hyphae colonize the cortex, spreading directly from cell to cell. These f~~Features of the fungi described here are consistent with the *Paris* morphological type. However~~, the presence of highly ramified arbuscules is commonly referred to as a characteristic of the *Arum* type (Smith and Read, 2010). Morphological variability observed in the AM fungi described is~~, it has been shown that the morphological types vary in a continnum depending on a series of factors (e.g. host plant tissues, taxa involved; environment; Dickson 2004; Van Aarle et al. 2004; Kubota et al. 2005), rendering this character merely descriptive and of relative use for the classification of AM fungi~~ consistent with previously observed morphologies varying in a continuum for a single fungus within the same host plant (Smith and Read 2010; Walker et al. 2018)~~. Besides, the distinction of these morphologies have been used in fossil comparisons (Harper et al. 2015; Harper et al. 2016; Walker et al. 2018).

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Systematic affinities of the root nodules and the fungi

The root nodules ~~herein described~~described here are characterized by a central vascular cylinder surrounded by an endodermis, followed by several layers of cortex surrounded by an epidermis; spherical to spheroidal three dimensional morphology; and emergence at right angle from the parental root in variable patterns along the main axis. These are features shared by extant Araucariales, which includes the Podocarpaceae and Araucariaceae (see comparisons in Table 1). Additional vegetative and reproductive organs assignable to Araucariaceae and Podocarpaceae are found in the La Matilde Formation (e.g., wood, leafy branches, cones; Gnaedinger 2007; Zamuner and Falaschi 2005; Falaschi et al. 2011; Kloster and Gnaedinger 2018; Escapa et al. in prep). Further refinement of the systematic affinities of the nodules based on the morphological characters preserved, especially considering morphological variability known to be present in fossil and extant Podocarpaceae and Araucariaceae, is not possible (Spratt 1912; Dickie and Holdaway 2011; Tables 1,2). Considering the presence of both podocarpaceous and araucariaceous organs in the chert blocks studied and the little morphological variation observed among the mycorrhizal nodules it remains possible that more than one taxa is present.

Based on phylogenetic analysis of combined molecular and morphological data, several families have been recognized among the former Glomeromycota (Schüßler et al. 2001; Spatafora et al. 2016): Paraglomeraceae, Archaeosporaceae, Geosiphonaceae, Ambisporaceae, Glomeraceae, Claroideoglomeraceae, Pacisporaceae, Gigasporaceae, Pacisporaceae, Sacculospporaceae, Acaulosporaceae and Diversisporaceae (see Schüßler and

Walker 2010; Redecker et al. 2013; http://www.amf-phylogeny.com). Differences mainly in spore type formation, spore wall structure, presence of vesicles, and even ecology, exclude affinities of the fungi described here with several different groups (see Table 3). Particular morphological characters suggest that these fossils most probably affinities are with the Paraglomeraceae. Diagnostic characters supporting affinities with the Paraglomeraceae include ~~Glomeromycotan familes can be distinguished morphologically; however, genera from different families are not always morphologically distinguishable (Schüßler and Walker 2010). Fungi forming arbuscular mycorrhizae in the root nodules herein described are characterized by globose to subglobose glomoid spores, which is shared with the Glomeraceae, Paraglomeraceae, Diversisporaceae, Claroideoglomeraceae, Ambisporaceae, Pacisporaceae and Geosiphonaceae. Affinities with the Claroideglomeraceae, Pacisporaceae and Geosiphonaceae are discarded based on differences in the spore wall structure and spore development and germination (Schüßler 2002; Schüßler and Walker 2010; Ohel and Sieverding 2004). The Ambisporaceae are characterized by the development of glomoid or acaulosporoid spores, or both; they also develop vesicles, though some species do not form vesicles (Walker et al. 2007). Species of the Diversisporaceae also develop either glomoid or acaulosporoid spores, sometimes also having vesicles and auxiliary cells (Walker and Schüßler 2004). The Glomeraceae typically develop glomoid spores, but are regarded as producing vesicles (Pirozynski and Dalppé 1989; Morton and Benny 1990), which is a feature not observed in the fungi herein described. The Paraglomeraceae are also characterized by the development of glomoid spores and lack of vesicles; vesicles have been observed only in the~~

~~species *Paraglomus brasilianum*, although, these have also been regarded as being intra-rhizal spores (Morton and Redecker 2001). Based on the presence of~~ arbuscules attached to narrow trunks (<4 µm in diameter), development of intra-rhizal spores and intracellular hyphae frequently ~~coiling 3-10 µm wide the fossil fungus described here is most similar to the Paraglomeraceae~~coiling. However, *Paraglomus* spores have a three-layered wall, whereas in our specimens, the wall is comprised by two layers. Nonetheless, the outer wall of the spores in *Paraglomus* has been mentioned to disintegrate at senescence, resulting in a two-layerd spore wall and thus, comparable to that displayed by the fossils described here (Morton and Redecker 2001). Alternatively, affinities of the fossil with Glomeraceae are also possible, being different mainly based on the absence of vesicles; additionally, the Glomeraceae commonly develop straight hyphae, whereas, the fossil most frequently shows coiled hyphae. These same features also ~~makes~~make the fossil somewhat comparable to Diversisporaceae and Ambisporaceae, although these two families develop acaulosporoid spores, which have not been observed in the fossil.

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Fossil conifers with arbuscular mycorrhizal fungi ~~conifers~~

~~Fossil examples of mycorrhization in conifers have been reported from the Triassic to the Eocene, including very scarce examples of arbuscular mycorrhizae in voltzialean, taxodiaceous and araucarialean conifers, and one example of ectomycorrhizal symbiosis in pinales (Strullu-Derrien and Strullu 2007 and citations therein; Schwendemann et al. 2011; Harper et al. 2015).~~ The oldest record of nodular roots colonized by arbuscular mycorrhizal ~~conifers~~ fungi is ~~comprised by~~

~~nodular roots and rootlets of voltzialean conifers~~ from the Triassic of Antarctica (Schwendemann et al. 2011; Harper et al. 2015). In this case, both the rootlets and the root nodules of *Notophytum krauselli* are colonized by serveral fungal estructures (i.e. ~~Hyphal~~ hyphal coils, ~~and~~ arbuscules, extra-rhizal hyphae, ~~occur in the cortical tissue of rootlets and nodules of *Notophytum*, as well as extra-rhizal hyphae,~~ vesicles and spores; ~~(~~Schwendemann et al. 2011; Harper et al., 2015). However, fungal structures found in the nodules are different from those found in the secondary rootlets, suggesting different arbuscular mycorrhizal symbiosis occurring in different organs of the same plant root system (Harper et al. 2015). Other examples of conifers showing root nodules ~~apparent mycorrhization in conifers come~~ are from different Cretaceous localities from the Southern Hemisphere (Cantrill and Douglas 1988; Cantrill and Falcon-Lang 2001; Banerji and Ghosh 2002). The lack of anatomical preservation in these nodules found as impressions, compressions and molds prevents any fungal association to be observed.~~Symbiosis is inferred based on the presence of root nodules preserved as impressions, compressions and molds, but none fungal structure is actually preserved (Cantrill and Douglas 1988; Cantrill and Falcon-Lang, 2001; Banerji and Ghosh, 2002).~~ Numerous affinities were discussed for these Cretaceous conifer nodule-bearing roots based on the association with other organs, including affinities with the Podocarpaceae, Araucariaceae, Sciadopityaceae, and even Cupressaceae. ~~Also, anatomically preserved arbuscular mycorrhizae were described in roots of *Metasequoia* from the Middle Eocene of Princeton Chert, British Columbia (Stockey et al. 2001). Therefore, t~~This new record of arbuscular mycorrhizal in root nodules with affinities with the Araucariales from the Jurassic of

Patagonia fills a gap in the geological history of AM fungi and further documents morphological diversity and ecological preferences of glomeromycetes through time.

Mycorrhizal nodules and ecology

The exceptional preservation of the conifer nodular roots of the 'Cañadón Nahuel' locality can be related to the mode of preservation typical of hot-spring settings, where plants and other organisms become immersed in a mineral-saturated solution that ~~slowly~~ gradually transform them into fossils (Channing and Edwards 2004; Hellawell et al. 2015). The presence of fungal structures inside the cortex of these underground organs indicates that the fossilization process of these organisms must have occurred while they were alive or soon after their death (Channing and Edwards, 2004). Such fossilization process consisted in permineralization by silica, which is a characteristic previously observed in 'La Bajada' and 'San Agustín' hot-spring localities also of the La Matilde Formation (García Massini et al. 2016). Moreover, based on their distribution and textural and fossiliferous content, the plant-rich cherts of the 'Cañadón Nahuel' locality are related to the distal areas of siliceous sinter geothermal system, potentially analogue to modern Yellowstone National Park geothermally influenced environments in the USA (Channing et al 2009a,b). Root nodules have also been preliminary described in the above mentioned 'San Agustín' and 'La Bajada' localities (see supplemental material; García Massini et al., 2013, 2016). These root nodules share the general morphology and anatomy of the specimens described here, though the best preserved examples of arbuscular ~~mycorrhizae~~

mycorrhizal colonization are those from 'Cañadon Nahuel'.

~~The distribution of organisms, especially of the vegetation, in the geothermal wetland and distal environments of hot-spring systems is limited by the unusual chemical and physical characteristics (Walter and Des Marais 1993; Channing et al. 2011; Channing and Edwards 2013; Channing 2017). These environments are often dominated by high temperatures, elevated salinity, extreme pH values, and presence of phytotoxic elements such as heavy- and transition-metals and metalloids (Channing and Edwards 2009; Channing 2017). In the distal areas of hot-spring systems, osmotic and chemical stresses also limit organisms' establishment (Channing and Edwards 2013; Channing 2017). In hot springs, where specialized organisms inhabit, fungal symbioses are probed to confer host plants stress tolerance to extreme physico-chemical parameters and, at the same time, positively influence fungal distribution along the geothermal gradient (Redman et al 2002; Rodriguez and Redman 2008; Rodriguez et al. 2008; Bunn and Zabinsky 2003). Examples of arbuscular mycorrhizal symbiosis in plants growing in geothermal soils have been reported for Yellowstone National Park (Bunn and Zabinsky 2003; Appoloni et al. 2008; Bunn et al. 2009). This stress tolerance through symbiosis in plants has also been reported for other environments (*e.g.*, salt marshes) with similar chemical stresses (Rodriguez et al. 2004; Rodriguez et al. 2008; Wilde et al. 2009).~~

Presence of arbuscular mycorrhizal fungi is not needed for the development of the root nodules in extant Araucariales, as they are indeed part of the root system ontogeny (Baylis et al. 1963; Baylis 1969), however, these organs are hypothesized to increase the volume of fungal colonization at a lower energy cost

than having an extensive root system and therefore maximize mineral nutrient acquisition efficiency (Russell et al. 2002; Dickie and Holdaway 2011). Although the role of the mycorrhizal nodules in the extant Araucariales is not yet completely understood, these organs are ~~believed~~ thought to be ecologically significant with respect to uptake of water and nutrients, especially phosphorous and nitrogen; additionally, establishment of seedlings in nutrient-poor environments has also been suggested to be influenced by the development of mycorrhizal nodules (Russel et al. 2002; Padamsee et al. 2016; Dickie and Holdaway 2011 and citations therein). ~~Presence of arbuscular mycorrhizal fungi in the root nodules of the Araucariales of the 'Cañadón Nahuel' locality may have played a key role for the colonization of the hostile distal areas of the hot-spring environments. Further, the presence of mycorrhizal nodules might have been crucial for the establishment conifers in the Jurassic waterlogged hot-spring settings. Similarly, it has been hypothesized that AM fungi are crucial for the development of podocarps in hot-spring related wetlands (Russel et al. 2002).~~ Systematic and taxonomical refinement to the family level of the conifers of 'Cañadón Nahuel' and further characterization of the paleo-environments where this plants used to live should enrich what can be grasped from these fossils on the evolution and significance of the AM symbiosis in the Jurassic Araucariales of Patagonia.

CONCLUSIONS

We present the first record of structurally preserved arbuscular mycorrhizal fungi colonizing root nodules~~e~~ from the Jurassic, partially filling a gap in the Mesozoic record of conifer mycorrhizal nodules, and extending the record

519 geographically.

520 The mycorrhizal nature of the fungus~~nodules~~ is demonstrated by the
521 presence of several fungal structures in a delimited zone of the nodule's cortex,
522 especially important is the presence of hyphal coils, arbuscules and glomoid
523 spores. ~~These mycorrhizal organs would have played a crucial role in the~~
524 ~~establishment of conifers in the distal areas of the hot-spring systems.~~

525 The nodules have affinities with the Araucariales clade of conifers, however, it
526 is not possible to constrain the affinities of these organs further based on the
527 anatomical evidence preserved. Nonetheless, the presence of these structures is
528 important as they expand our knowledge on the morphology and ecology of these
529 conifers during the establishment of the modern conifer families in the Jurassic.
530 They also open the possibility to work on whole-plant concept reconstructions in
531 the future.

532

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TABLES AND FIGURES CAPTIONS

Table 1. Comparisons of 'Cañadón Nahuel' locality specimens of root nodules and the root nodules of extant Podocarpaceae and Araucariaceae species. AMF = arbuscular mycorrhizal fungi; PS = parallel series; LS = longitudinal series; VC = vascular cylinder; MR = main root; En. = endodermis; C. = cortex; E. = epidermis; Ex = exodermis; VS = vascular strand; P = Present; A = absent; Sc. = Scattered; ? = Missing information

Information taken from:

*a Breuniger et al 2000

*b McGee 1990

*c Godoy and Mayr 1989

*d Padamsee et al 2000

*e Russel et al 2002; Dickie and Holdaway 2011

*f Leone et al 2008

Table 2. Fossil record of conifer root nodules. Modified from Schwendemann et al. 2011.

AMF = arbuscular mycorrhizal fungi; P = preserved; NP = not preserved; ? = missing information

Table 3. Comparisons within families of the former Glomeromycota that form glomoid spores.

G = glomoid; Ac. = acaulosporoid; Occ. = occlusion; P = present; A = absent; ? = missing information or not clear

*a Schußler and Walker 2010

*b Ohel and Sieverding 2004

*c Schußler 2002

*d Walker et al 2007

*e Walker and Schußler 2004

*f Morton and Benny 1990

*g Morton and Redecker 2001

*h Redecker et al 2013

FIGURE 2. Thin sections of chert blocks from 'Cañadón Nahuel' locality, MPM-Pb-16039-16041. – a. General view of chert block showing conifer roots immersed in the matrix. Some nodules in young fine are colored with pink sof-light. – b. Oblique-cross section of conifer diarch root. Note secondary xylem (2ºX), cortex (C) and apparent periderm (Pd). – c. Oblique-longitudinal section of conifer root showing secondary xylem (2ºX) and cortex (C), periderm not observed. – d and –

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873 e. Detail of secondary xylem. Note araucarian pitting in the walls of the tracheids. –
874 f. General view of nodular root with spherical to spheroidal nodules, disposed with
875 any apparent pattern. Scale bar for b and c = 0.5 mm; for d and e = 10 µm; for f =
876 100 µm.
877
878 FIGURE 3. Thin sections of chert blocks from 'Cañadón Nahuel' locality, MPM-Pb-
879 16033;16039-16040. – a. General view of 'beaded' root with nodules, nodules
880 disposed alternately. – b. Cross section of a nodule, showing vascular trace (VT)
881 emerging from the parental root. – c. Oblique-longitudinal section of root, with
882 lateral root nodule emerging at right angle from the main axis. – d. Cross section of
883 a nodule surrounded by the main root cortex, showing general anatomy of the
884 nodule. Note vascular strand (VS), endodermis (En), Cortex (C) and epidermis (E).
885 Detail of vascular strand and endodermis inside box, scale bar = 20 µm. – e.
886 Detail of root nodule showing resurgent growth, new cortex (NC) developing inside
887 the cortex of the older nodule (OC). – f. General view of root hairs. Scale bar for –
888 a. 0.5 mm; for b, c, d, e and f = 100 µm.
889
890 FIGURE 4. Thin sections of chert blocks from 'Cañadón Nahuel' locality, MPM-Pb-
891 16040-16041. – a. General view of root nodule. Pink coloration indicates the
892 presence of hyphal coils. – b. Detail of a coenocytic hyphal coil showing knobs. – c.
893 Detail of coenocytic hyphae showing Y-branching. – d. Detail of coenocytic hyphae
894 showing branching at right angle. – e and f. Detail of worn out hyphael intruded by
895 a septate fungus (white arrow heads). Scale bar for a = 100 µm; for b, c, d, e and f
896 = 10 µm.

FIGURE 5. Thin section of chert block from 'Cañadón Nahuel' locality, MPM-Pb-16039, and thin section of extant Podocarpaceae *Podocarpus totara* root nodule (Images by Larry Peterson and Lewis Melville, from Dickie & Holdaway, 2011) – a. Longitudinal section of fossil root nodule showing zone of the cortex occupied by arbuscules (delimited by dotted line) – b. Longitudinal section of extant root nodule showing zone of the cortex occupied by arbuscules (delimited by dotted line). – c. Detail of arbuscules in fossil root nodule. – d. Detail of arbuscules in extant root nodule. Scale bar for a and b = 100 µm; for c = 20 micras; for d = 50 µm

FIGURE 6. Thin section of chert block from 'Cañadón Nahuel' locality, MPM-Pb-16039. Diversity of arbuscules partially occupying cortical cells of root nodules. Scale bar for all = 10 µm.

FIGURE 7. Thin section of chert block from 'Cañadón Nahuel' locality, MPM-Pb-16039. – a. Root nodule with intra-rhizal glomoid spores. – b. Glomoid spore associated to root nodule. Note connecting hypha (arrowhead). – c. Glomoid spore with multiple hyphal attachments, some of which connect it to the root nodule. – d. Magnification of previous image showing detail of entry point with penetrating hyphae and development of coil. – e. Detail of intra-rhizal globose spore in decaying root, showing two-layered wall, simple subtending hypha and occlusion plug. – d. Detail of extra-rhizal globose spore, showing two-layered wall, simple subtending hypha, and occlusion.

SUPPLEMENTAL MATERIAL

Appendix 1. – A1. Diversity of young fine roots and nodules from different localities of the La Matilde Formation, Deseado Massif, Santa Cruz Province, Argentina. – a, b and c. 'Cañadón Nahuel' locality. – d. 'San Agustín' locality. – e. 'La Bajada' locality. Scale bar for a and e = 0.5 mm; b and d = 0.25 mm; c = 0.1 mm

Appendix 2. – A2. Further images of arbuscules inside cortical cells of root nodules specimens of the 'Cañadón Nahuel' locality. Scale bar for all = 10 µm

Appendix 3. – A3. Sequence of original images used to obtain the final composition of the nodules endodermis. Scale bar for all = 20 µm.

Appendix 4. - A4I and A4II. Sequences of original images used to obtain the final composition of coiling hypha. Scale bar for all = 10 µm.

Appendix 5. – A5. Sequence of original images used to obtain the final composition of nodule's cortical cells occupied by arbuscules. Scale bar for all = 20 µm.

Appendix 6. - A6I, A6II, A6III. Sequences of arbuscules at different focal planes. Scale bar for all = 10 µm.

Appendix 7. - A7I. Sequence of original images used to obtain the final composition of a root nodule occupied by intra-rhizal glomoid spores. Scale bar for all = 100 µm. – A7II. Sequence of original images used to obtain the final composition of a germinating spore with hyphae getting attached to the epidermis of a root nodule. Scale bar for all = 20 µm. – A7III. Sequence of original images used to obtain the final composition of a hypha penetrating the epidermis of a root nodule. Scale bar for all = 10 µm. – A7IV. Sequence of original images used to obtain the final composition of an intra-rhizal glomoid spore. Scale bar for all = 10 µm.

~~FIGURE 1. Diversity of young fine roots and nodules from different localities of the La Matilde Formation, Deseado Massif, Santa Cruz Province, Argentina. – a, b and c. 'Cañadón Nahuel' locality. – d. 'San Agustín' locality. – e. 'La Bajada' locality. Scale bar for a and e = 0.5 mm; b and d = 0.25 mm; c = 0.1 mm~~

~~FIGURE 2. Further images of arbuscules inside cortical cells of root nodules specimens of the 'Cañadón Nahuel' locality. Scale bar for all = 10 µm~~

Table 1

Conifer species	Nodules					AMF	
	Arrangement	Morphology	Size mm	General anatomy	Root hairs	Outer cortex	Inner cortex
Araucaria angustifolia *a	?	Spheroidal	?	?	?	Penetration hyphae	Arbuscules, vesicles
Araucaria cunninghami *b	?	Spherical and conical	?	Central VC connected to MR. / En. / C. / E.	?	Scattered hyphae	Arbuscules
Araucaria araucana *c	PS	Spheroidal	0.55	Central VC connected to MR. / En. / C. / E. Ex (in older nodules)	P (young nodules)	Mainly free.	Abundant arbuscules, vesicles
Agathis australis *d	Irregularly spaced	Spherical. Beaded nodules.	≤1	Central VC connected to MR. / En. / C. / E. VS = half length of the nodule	?	Scattered hyphae	Coiled hyphae, arbuscules, vesicles
*Agathis robusta*b	?	Spherical and conical	?	Central VC connected to MR. / En. / C. / E.	?	Scattered hyphae	Arbuscules
Wollemia nobilis *b	?	Spherical and conical	?	Central VC connected to MR. / En. / C. / E.	?	Scattered hyphae	Arbuscules
Dacrycarpus dacrydioides *e	2 or 4 LS	Spherical	?	Central VC connected to MR. /En. / C. / E. VS = length of the nodule	A	Mainly free. Scattered hyphae (below entry point)	Arbuscules, vesicles
Dacrydium cupressinum *e	2 or 4 LS	Spherical	?	Central VC connected to MR. / En. / C. / E. VS = half length of the nodule	A	Mainly free. Scattered hyphae (below entry point)	Arbuscules, vesicles
Dacrydium fonckii *c	?	Spherical	?	?	?	?	Abundant arbuscules, scattered vesicles
Podocarpus nubigena *c	PS	Spherical	1.2	?	P	?	Abundant arbuscules
Podocarpus parlatorei *f	?	Spherical	?	?	?	?	Arbuscules, vesicles
Podocarpus salignus *c	2 or 4 LS	Spherical	0.7	?	P	?	Arbuscules, vesicles
*Prumnopitys ferruginea*e	2 or 4 LS	Spherical	?	Central VC connected to MR. / En. / C. / E. VS = half the length of the nodule	P	Mainly free. Scattered hyphae (below entry point)	Arbuscules, vesicles
Prumnopitys taxifolia *e	2 or 4 LS	Spherical	?	Central VC connected to MR. / En. / C. / E. VS = half the length of the nodule	P	Mainly free. Scattered hyphae (below entry point)	Arbuscules, vesicles
Prumnopitys andina *c	?	?	1.1-1.5	?	Sc.	?	Arbuscules, vesicles
Saxegothaea conspicua *c	PS	?	0.5-1.0	?	P	?	Arbuscules, vesicles
'Cañadón Nahuel' specimens	Variable	Spherical or Spheroidal	0.8	Central VC connected to MR. / En. / C. / E. VS = half the length of the nodule	P (occasionally)	Mainly free. Scattered hyphae (below entry point)	Coiled hyphae, arbuscules, spores

Age	Locality	Preservation	Nodules	Affinities	AMF
Middle Triassic	Antarctica	Permineralization	Prolate, spheroidal 1.1 x 0.8 mm	Voltziales	P
Middle Jurassic	Patagonia, Argentina	Permineralization	Spherical, spheroidal 0.8 mm	Araucariales	P
Cretaceous	Australia	Compresions/Impressions	?	?Taxodiaceae	NP
	Antarctica	Compresions/Impressions	Spherical ≤ 2 mm	?Podocarpaceae	NP
	India	Molds	Spherical, oval 1-2.5 x 1-2 mm	?Podocarpaceae ?Araucariaceae	NP

Family	Spore type	Glomoid spore wall structure and occlusion	Vesicles	Mycelium	Observations
Claroideoglomeraceae *a,h	G	2 layers (apparent 'endospore'). Occ.: Septum	?	?	?
Pacisporaceae *a,b,h	G	Two wall groups. Occ.: Septum	P (some species)	?	?
Geosiphonaceae *c,h	G	3 layers Occ.: amorphous plug.	?	Sparsely septate at senescence	Association with cyanobacteria
Ambisporaceae *d,h	G/Ac.	2 layers Occ.: Open-pored or Septum	P/A	?	?
Diversisporaceae *e,h	G/Ac.	3 layers	P/A	?	With or without auxiliary cell
Glomeraceae *f,h	G	3 layers Occ.: variable	P	Narrow trunks. Coiling rare	?
Paraglmoeraceae *g,h	G	3 layers Occl.: Septum?	P?/A	Narrow trunks. Coiling frequent	Intra-rhizal spores?

Figure 1

Click here to access/download;Figure;Figure1.tif

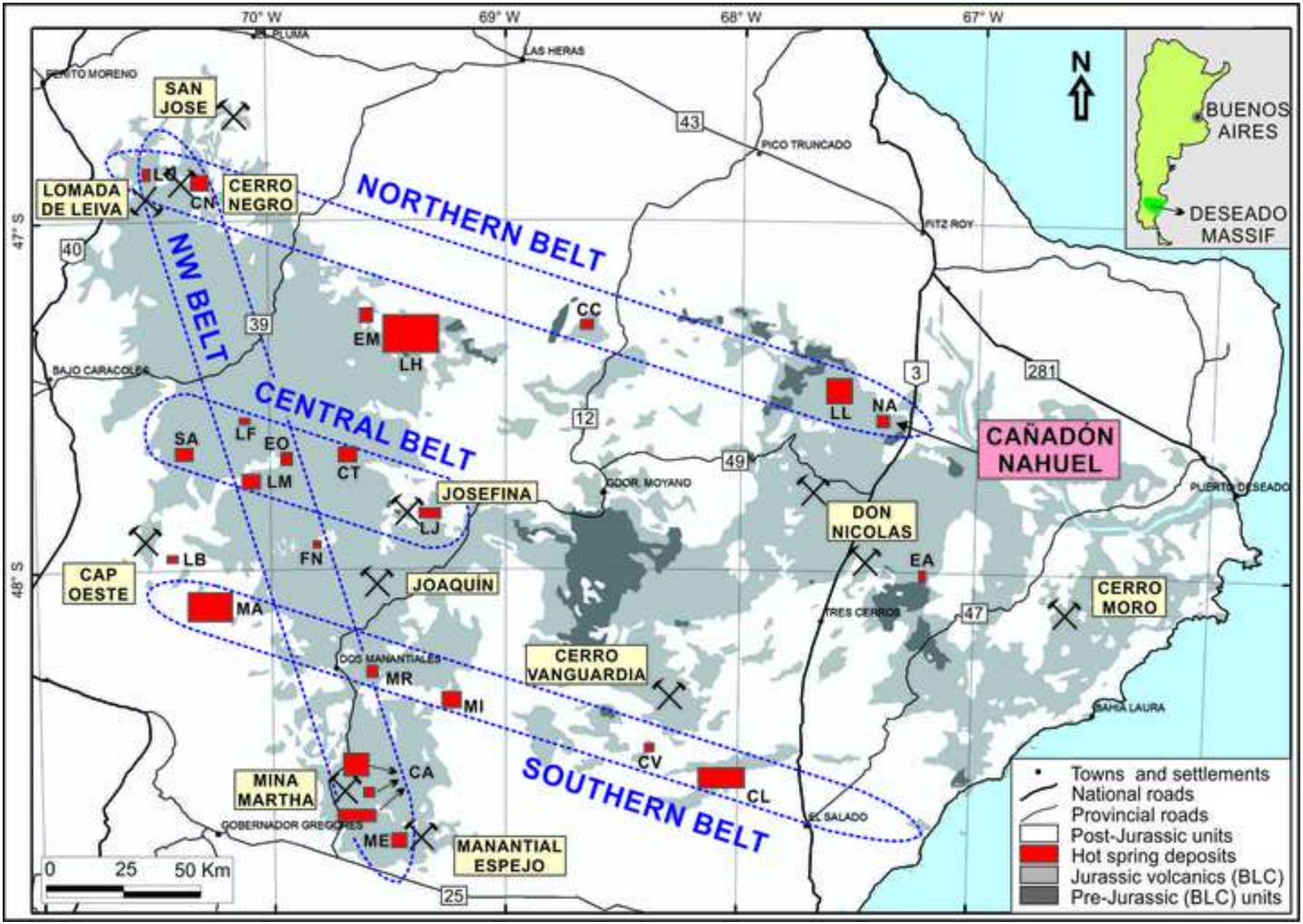

Figure 2

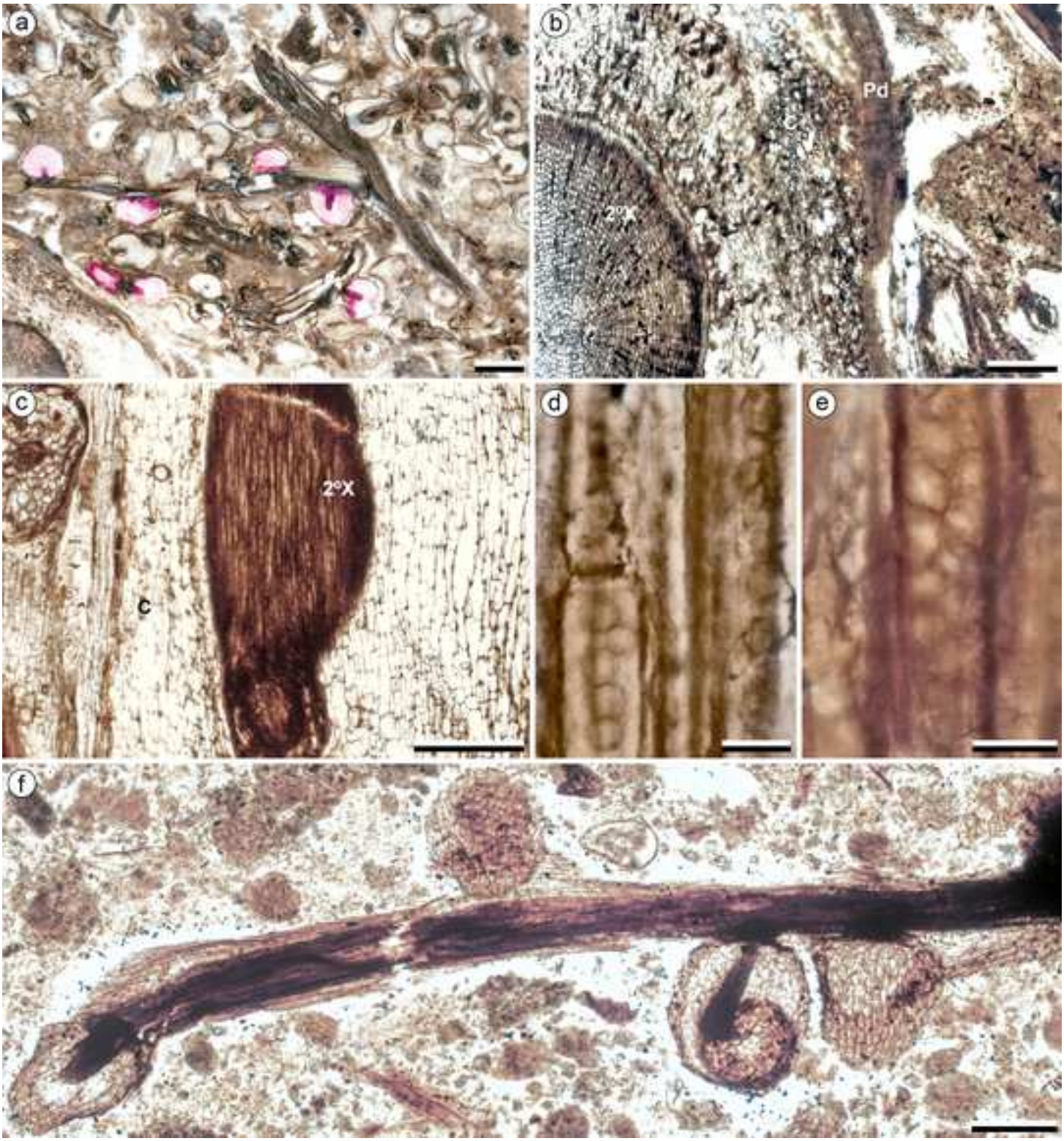

Figure 5

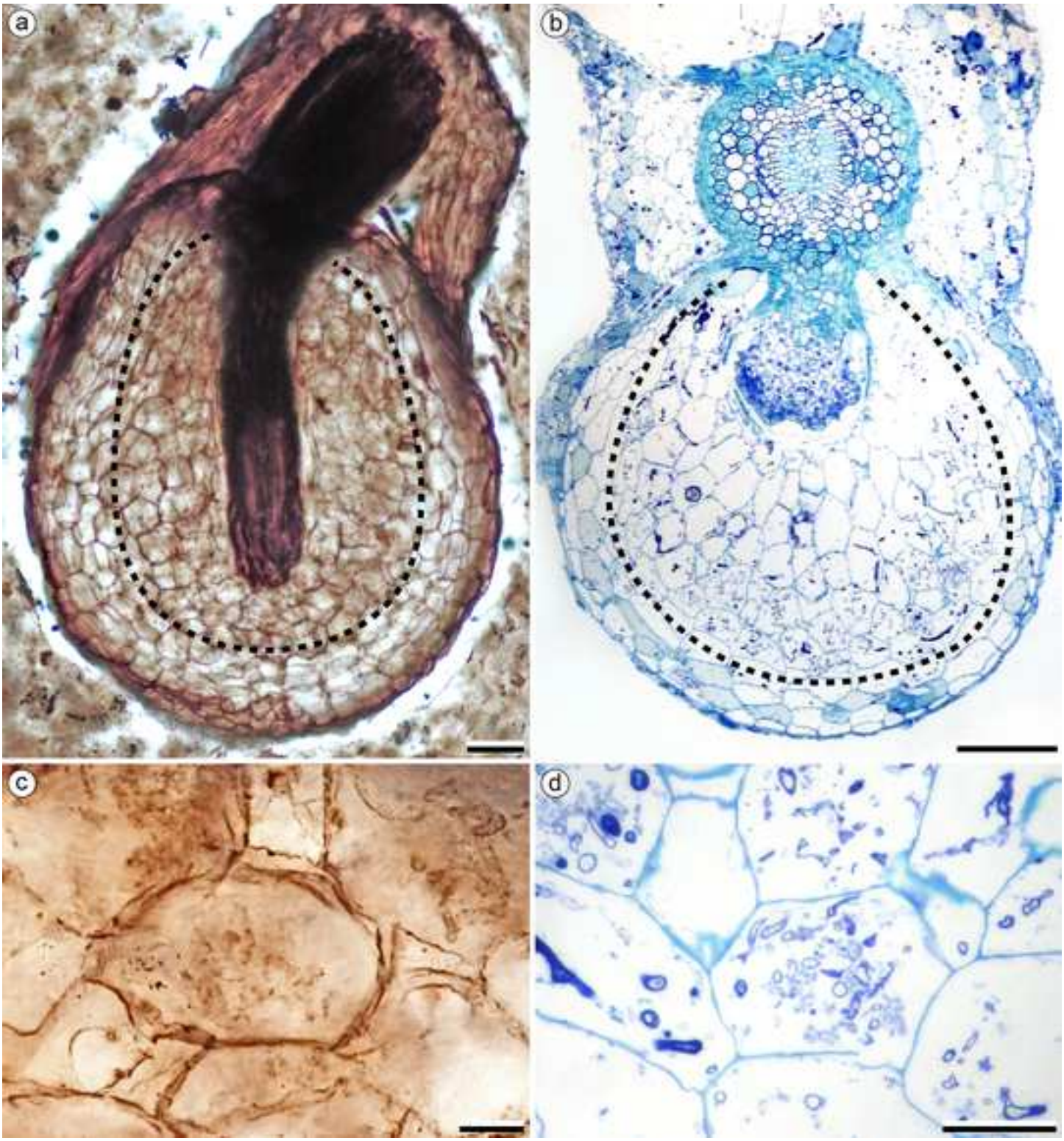

Figure 6

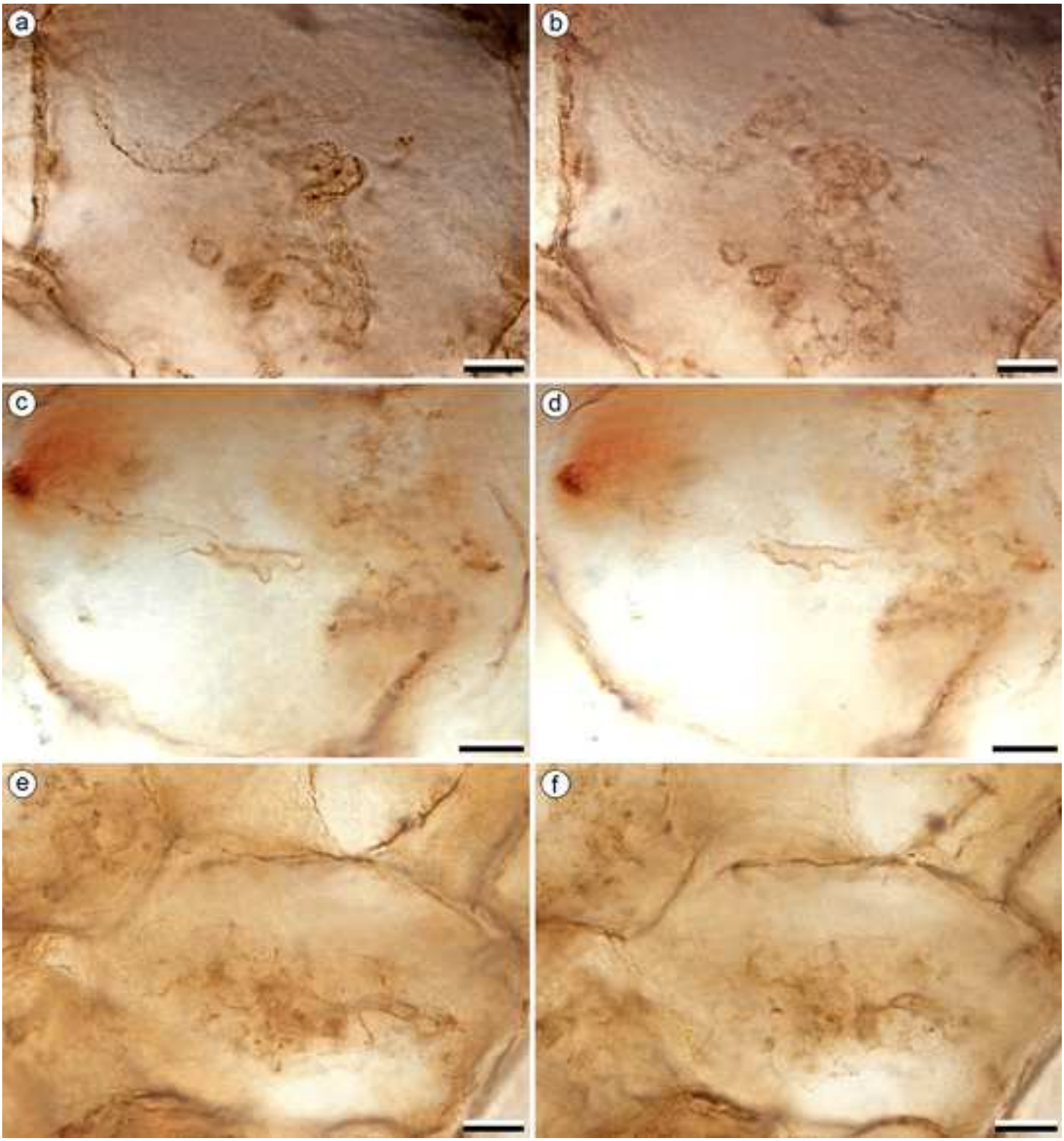

Figure 7

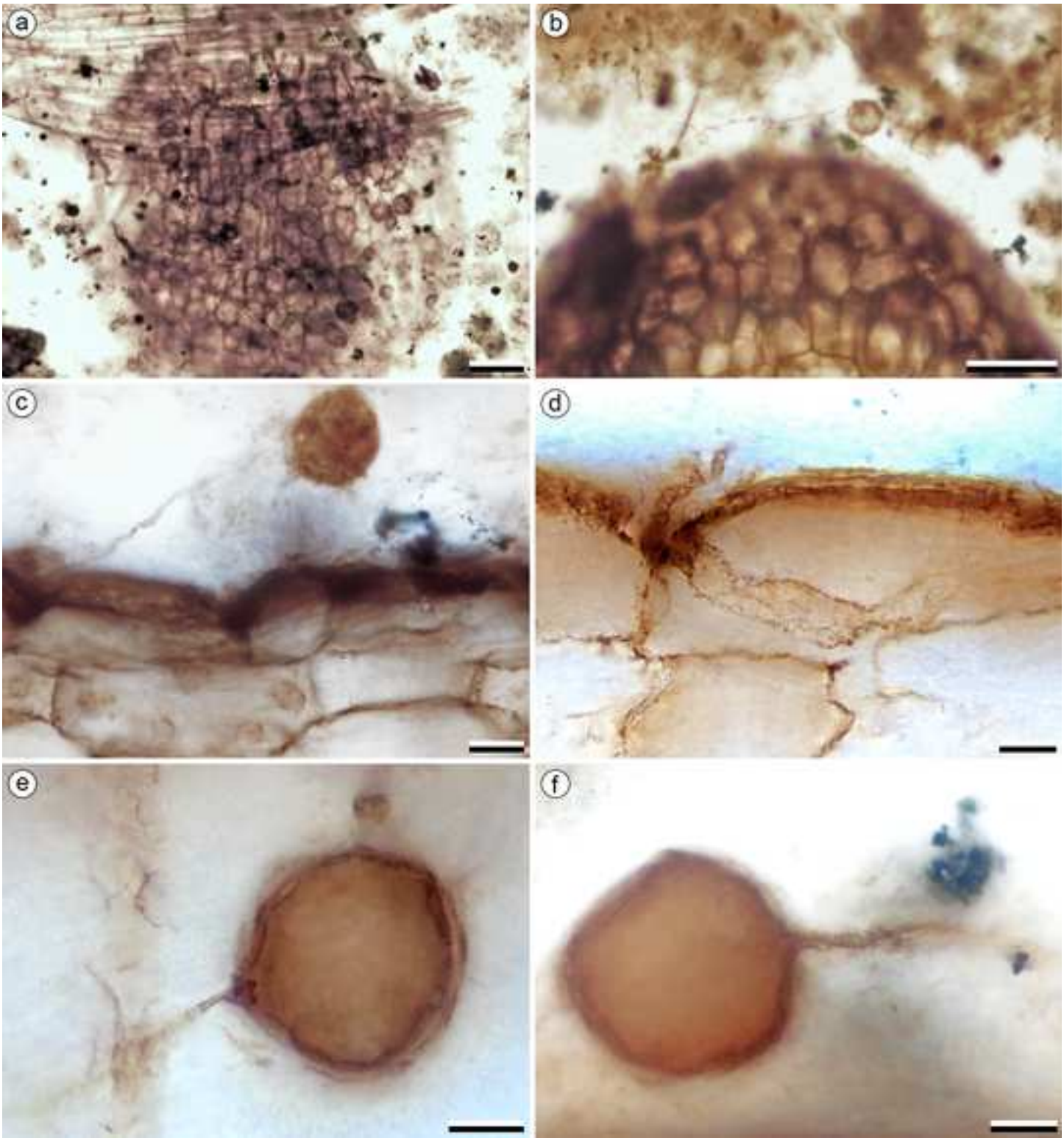

Figure 3

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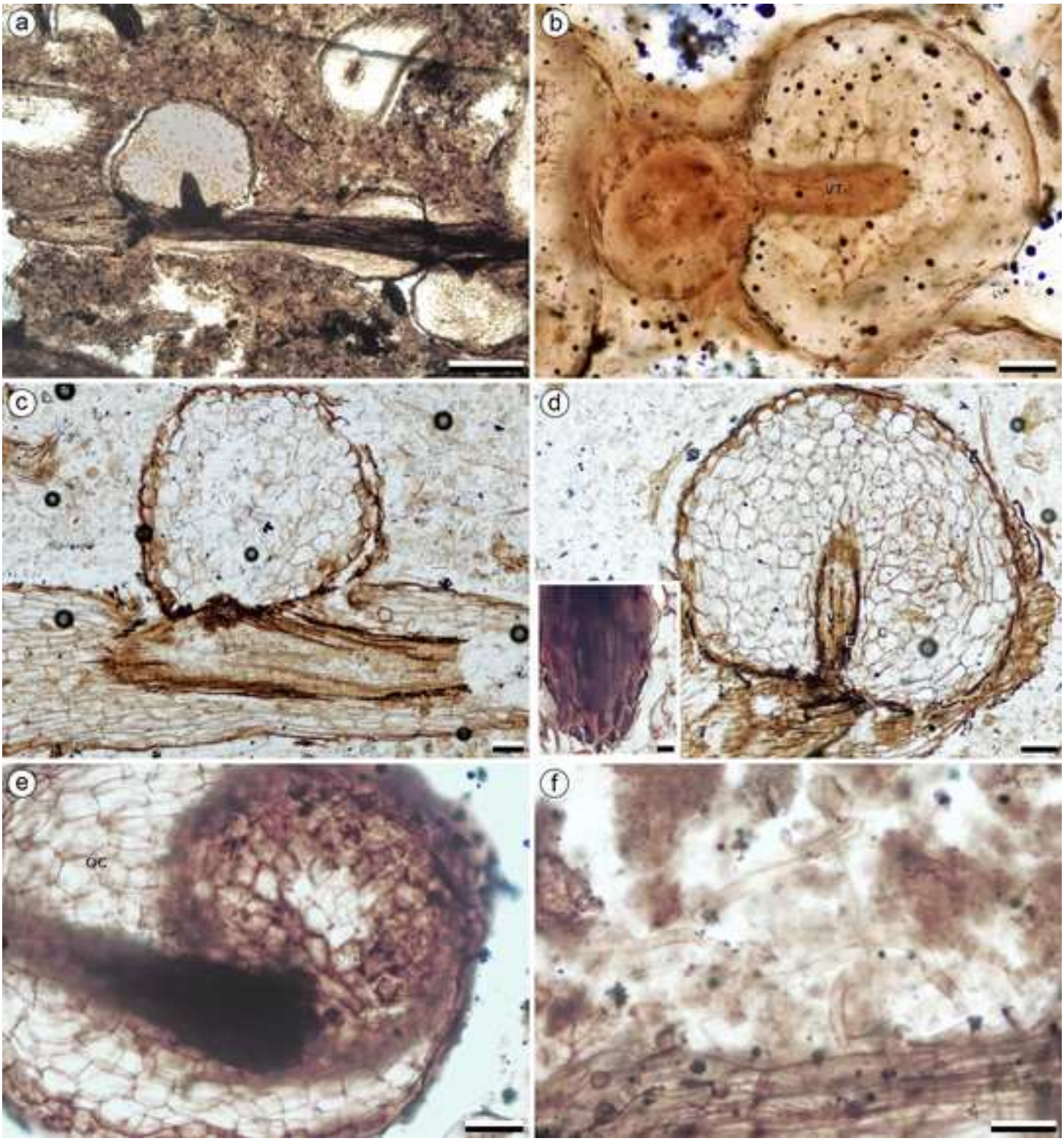

Figure 4